autoreactive T cells of these patients, to the autoantigenic proteins in the articular cartilage under attack and other self antigens which display the identified MHC Class II binding T cell epitopes characterized or mimicked by the amino acid sequences of one or more of the peptides according to the invention. The induced tolerance thus will lead to a reduction of the local inflammatory response in the articular cartilage under attack.

Very suitable peptides to be used in a pharmaceutical composition according to the invention are the peptides comprising the YKL-39 (268-276) or the YKL-39 (266-278) peptide flanked by sequences up to a total length of 55 amino acids. More preferably the peptides have a length of 25 amino acids. Even more preferably the amino acid sequence of the peptides is FTLASAETT (SEQ ID NO: 1) or HSFTLASAETTVG (SEQ ID NO: 2).

The peptides according to the invention have the advantage that they have a specific effect on the autoreactive T cells thus leaving the other components of the immune system intact as compared to the nonspecific suppressive effect of immunosuppressive drugs. Treatment with the peptides according to the invention will be safe and no toxic side effects will occur.

Systemic immunological tolerance can be attained by administering high or low doses of peptides according to the invention. The amount of peptide will depend on the route of administration, the time of administration, the age of the patient as well as general health conditions and diet.

In general, a dosage of 0.01 to 10000 µg of peptide per kg body weight, preferably 0.05 to 500 µg, more preferably 0.1 to 100 µg of peptide can be used.

Pharmaceutical acceptable carriers are well known to those skilled in the art and include, for example, sterile saline, lactose, sucrose, calcium phosphate, gelatin, dextrin, agar, pectin, peanut oil, olive oil, sesame oil and water. Other carriers may be, for example MHC class II molecules, if desired embedded in liposomes.

In addition the pharmaceutical composition according to the invention may comprise one or more adjuvants. Suitable adjuvants include, amongst others, aluminum hydroxide, aluminum phosphate, amphigen, tocophenols, monophosphenyl lipid A, muramyl dipeptide and saponins such as Quill A. Preferably, the adjuvants to be used in the tolerance therapy according to the invention are mucosal adjuvants such as the cholera toxine B-subunit or carbomers, which bind to the mucosal epithelium. The amount of adjuvant depends on the nature of the adjuvant itself.

Legends to the figures

Figure 1

Figure 1a, b, c. Cross reactivity of three, different, HC gp-39-specific hybridomas (8B12,14G11,20H5) with YKL-39 (266-278)

(CVR0271B = HC gp-39 (263-275), KV0432B = YKL-39 (266-278), CC0332B = Chi (269-282), KV0431A = YKL-39 (262-274). HCDA.8B12.1D8, 14G11.1H7 and 20H5.4F6.2F6 are HLA-DRB1*0401-restricted hybridomas specific for HC gp-39 (263-275). Activation of T-cell hybridomas is expressed as IL-2 production.

Figure 2 In vivo tolerization with HC gp-39 (263-275) or YKL-39 (266-278)

Balb/c mice were tolerized by intranasal application of 50, 10 or 2 microgram of HC gp-39 (263-275) or YKL-39 (266-278) followed by immunization with HC gp-39 (263-275). Mice that were pretreated with saline or that were left untreated were included as controls.

C2

Examples

Example 1 Alignment of sequences

The human chondrocyte protein, YKL-39 shares significant sequence identity with HC gp-39 (YKL-40). Another homologue of HC gp-39 is secreted by human macrophages and is termed chitotriosidase (Boot et al., 1995). The sequences corresponding to RSFTLASSETGVG (HC gp-39 (263-275), SEQ ID NO: 3) were identified as HSFTLASAETTVG (SEQ ID NO: 2) within the YKL-39 protein (266-278) and as RSFTLASSSDTRVG (SEQ ID NO: 4) within macrophage chitotriosidase (269-282) respectively (Table 1). Chi (269-282) contains the HLA-DRB1*0401 peptide binding motif which was previously used for selection of T-cell epitopes within proteins. In contrast, the YKL-39 (266-278) peptide does not contain this motif. All peptides were synthesized.

Table 1. Alignment of the HC gp-39 (263-278) sequence with the corresponding region in YKL-39 and macrophage Chitotriosidase.

HCgp-39 263-275	R S F T L A S S - E T G V G (SEQ ID NO: 3)
YKL-39 266-278	H S F T L A S A - E T T V G (SEQ ID NO: 2)
Chi (269-282)	R S F T L A S S S D T R V G (SEQ ID NO: 4)

Example 2 Binding of peptides to HLA-DRB1*0401

The peptides from example 1 were tested for binding the DRB1*0401-encoded molecules. HLA-DR4 (DRB1*0401) molecules were purified from the homozygous EBV-transformed human B lymphoblastoid cell lines Huly138IC2 and the competition peptide HLA-DR binding assay was performed basically as described by Verheijden et al., 1997. The affinity of a given peptide for binding DRB1*0401-encoded molecules was related to competition with a marker peptide. This relative binding affinity was defined as the peptide concentration at which the signal was reduced to 50% (IC50). The HA-F peptide is a positive control (Hemagglutinin 307-319; PKFVKQNTLKLAT; at position 309 Y is substituted by F; SEQ ID NO: 5). The peptide is known to have a high affinity for DRB1*0401 molecules.

C3

As expected, the Chi(269-282) peptide was found to bind with high affinity to DRB1*0401 (see table 2). The YKL-39 (266-278) peptide, which does not accommodate the effective DRB1*0401 peptide binding motif, bound with very high affinity to DR4 (B1*0401).

Table 2 Peptide binding to HLA-DRB1*0401-encoded molecules

		IC50 values		
peptide	batch	Exp.A	Exp.B	Exp.C
YKL39(262-274)	KV0431A	0.006	0.005	ND
YKL39(266-278)	KV432B	0.035	0.032	0.12
HCgp39(263-275)	CVR271B	ND	0.008	0.038
Chi(269-282)	CC0332B	0.053	0.11	0.16
HA-F	AE0690A	0.20	0.14	0.20

ND=not determined